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Identification of Blood Culture Isolates Directly from Positive Blood Cultures by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry and a Commercial Extraction System: Analysis of Performance, Cost, and Turnaround Time

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Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry represents a revolution in the rapid identification of bacterial and fungal pathogens in the clinical microbiology laboratory. Recently, MALDI-TOF has been applied directly to positive blood culture bottles for the rapid identification of pathogens, leading to reductions in turnaround time and potentially beneficial patient impacts. The development of a commercially available extraction kit (Bruker Sepsityper) for use with the Bruker MALDI BioTyper has facilitated the processing required for identification of pathogens directly from positive from blood cultures. We report the results of an evaluation of the accuracy, cost, and turnaround time of this method for 61 positive monomicrobial and 2 polymicrobial cultures representing 26 species. The Bruker MALDI BioTyper with the Sepsityper gave a valid (score, >1.7) identification for 85.2% of positive blood cultures with no misidentifications. The mean reduction in turnaround time to identification was 34.3 h ($P < 0.0001$) in the ideal situation where MALDI-TOF was used for all blood cultures and 26.5 h in a more practical setting where conventional identification or identification from subcultures was required for isolates that could not be directly identified by MALDI-TOF. Implementation of a MALDI-TOF-based identification system for direct identification of pathogens from blood cultures is expected to be associated with a marginal increase in operating costs for most laboratories. However, the use of MALDI-TOF for direct identification is accurate and should result in reduced turnaround time to identification.

Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has been routinely used for the identification of bacteria and fungi from agar culture in many centers in Europe and is increasingly used in North America and elsewhere for primary identification of microorganisms (1, 2, 25). MALDI-TOF instruments use an ionizing laser to vaporize the abundant structural elements (primarily ribosomal proteins) of bacteria and yeasts and analyze the weight and relative abundance of each particle to generate a spectrum. Spectra are compared to a computer database of reference or user-defined organism spectra, and identification is obtained by matching the most similar spectrum in the database to the unknown organism. Performance of the Bruker MALDI BioTyper has been extensively studied in multiple centers and confirms that reliable identification can be obtained for >95% of the isolates grown on solid media routinely encountered in the clinical laboratory (1, 4, 5, 8, 19).

More recently, protocols for the direct identification of pathogens from positive blood culture broths have been developed (3, 12, 16, 21, 22). Although blood culture broths are usually monobacterial (or monofungal) cultures, the presence of proteins from red cells, white blood cells, and serum interferes with the analysis by adding spectral peaks not found in the organism database. Furthermore, interfering substances such as charcoal (when present) and low organism numbers (as might be encountered with slow-growing or contaminating bacteria) present additional challenges in the use and interpretation of MALDI-TOF spectra for identification pathogens directly from positive blood cultures (24). As a result, many centers have developed in-house methods for preprocessing of blood cultures to optimize recovery of the bacterial proteome. More recently, a commercial kit (Bruker Sepsityper) has been released to simplify the processing steps required for the purification and extraction of the bacterial proteome from positive blood cultures. The system serves to facilitate preprocessing and minimize the impact of the interfering human proteome on the MALDI-TOF analysis. Here we report the performance of the Sepsityper system on the Bruker MALDI BioTyper for the direct identification of pathogens from blood cultures and a cost and turnaround time analysis of the results.

MATERIALS AND METHODS

Blood cultures. Blood was collected at the bedside and directly inoculated into BacT/Alert SA (aerobic culture) and/or SN (anaerobic) (bioMérieux, Marcy l'Etoile, France). Both bottle types are charcoal free. Bottles were loaded onto the a BacT/Alert instrument (bioMérieux, Marcy l'Etoile, France) and incubated. Bottles were incubated for up to 5 days, and when the operator was notified of a positive blood culture, a Gram stain was performed. All positive bottles were subjected to subculture and routine

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TABLE 1 Distribution of clinically significant isolates from blood cultures in Canada[a] and cost estimates of abbreviated[b] and automated biochemical panel identifications

Organism	% of isolates	Cost (U.S. dollars) of:		Weighted cost (U.S. dollars)		
		Abbreviated identification	Automated panel identification	Low	High	Best
Escherichia coli	23.7	0.25	5.50	0.059	1.304	0.184
Staphylococcus aureus	17.0	0.25	5.50	0.043	0.935	0.132
Coagulase-negative *Staphylococcus*	10.6	0.25	5.50	0.027	0.583	0.082
Klebsiella pneumoniae	7.3	NA[c]	5.50	0.402	0.402	0.402
Streptococcus pneumoniae	5.1	0.25	5.50	0.013	0.281	0.040
Enterococcus faecalis	4.6	0.25	5.50	0.012	0.253	0.036
Beta-hemolytic *Streptococcus*	3.9	0.25	5.50	0.010	0.215	0.030
Pseudomonas aeruginosa	3.8	0.25	5.50	0.010	0.209	0.029
Enterobacter cloacae	2.4	NA	5.50	0.132	0.132	0.132
Enterococcus faecium	1.8	0.25	5.50	0.005	0.099	0.014
Viridans group *Streptococcus*	1.6	0.25	5.50	0.004	0.088	0.012
Serratia marcescens	1.6	NA	5.50	0.088	0.088	0.088
Klebsiella oxytoca	1.6	NA	5.50	0.088	0.088	0.088
Candida albicans	1.4	0.25	5.50	0.004	0.077	0.011
Proteus mirabilis	1.1	0.25	5.50	0.003	0.061	0.009
Other	12.5	NA	5.50	0.688	0.688	0.688
Avg/isolate				1.58	5.50	1.98

[a] Data from national surveillance studies available at http://www.can-r.ca.
[b] Where available in accordance with CLSI document M35-A2.
[c] NA, not available.

identification, as well as direct identification with the Sepsityper and MALDI BioTyper (Bruker-Daltonics, Bremen, Germany). Briefly, 1 ml of a positive blood culture was placed in a tube with lysis buffer and centrifuged. Washing buffer was added and recentrifuged. Finally the pellet was resuspended and subjected to ethanol-formic acid extraction according to the manufacturer's instructions. The extract was spotted on the MALDI-TOF target, overlaid with matrix, and analyzed with BioTyper version 3.0 according to the manufacturer's instructions. The total hands-on time for the procedure was approximately 12 min. In accordance with the manufacturer's instructions, identification scores of >1.7 were considered valid to the genus level and scores of >2.0 were considered valid to the species level. For this study, MALDI-TOF identification was batched twice daily rather than performed in real time.

Conventional identification. Routine identification methods included a variety of commonly used rapid (coagulase, indole, latex agglutination, Lancefield typing) and standard (Vitek 2 and API) biochemical tests, depending on the organism isolated. Abbreviated identification methods as described in CLSI document M35-A2 (6a) were used when appropriate. Thermonuclease testing was used to presumptively identify *Staphylococcus aureus* from blood cultures containing Gram-positive cocci in clumps. Discordant identifications were further characterized by additional biochemical methods or 16S rRNA gene sequencing when identification was deemed to be clinically relevant (not for isolates assumed to be contaminants).

Turnaround time. Turnaround time was determined by two approaches. The first, reflecting an ideal situation, was use of the laboratory information system as the time elapsed between the automated instruments flagging a positive blood culture to the time of identification by one of the two methods. Second, we considered a more practical turnaround time analysis whereby all of the organisms with a MALDI-TOF score insufficient for definitive identification or culture of an organism that requires further confirmation (e.g., members of the *Streptococcus mitis* group) continued to require conventional identification. A paired *t* test was used to identify any statistically significant difference between the MALDI-TOF system turnaround time and the conventional identification.

Costs. Typical distribution of organisms identified from positive blood cultures were obtained from Canadian surveillance data (Table 1). An estimate of the cost of conventional identification was determined by assuming that the cost of a biochemical panel was $5.50 per unit and the cost of abbreviated identification methods (oxidase, indole, catalase, coagulase, germ tube, pyrrolidonyl arylamidase test, Lancefield typing, and bile solubility) was $0.25 per identification. Although costs of abbreviated identification may vary slightly, depending on lab practice and method, this represents an estimated average cost. In order to estimate costs associated with identification on the blood culture bench, we used typical distribution of organisms identified on the bench to derive the weighted cost of identification of each species identified from blood cultures. The weighted costs were then added to determine the average cost of an identification. Because laboratories may use different methods of identification, a range was created by applying three potential situations (Table 1). A low cost estimate for laboratories using only abbreviated identification methods for eligible organisms, a high cost estimate for laboratories using identification panels for all isolates, and a "best estimate" where we assumed that for eligible organisms, 90% of identifications could be achieved by using abbreviated methods of identification detailed in CLSI document M35-A2 and the remainder would require panel identification. All other organisms were assumed to require identification panels for definitive identification. The cost of the Sepsityper identification was estimated by using manufacturer information to be $5.15 per identification (includes the cost of the kit and the ethanol-formic acid extraction method). In order to account for the cost of Sepsityper identification failures that require additional workup from subculture, we added the cost of routine identification for these isolates. It was assumed that 14.8% of cultures could not be identified by the Sepsityper (derived from the present study) and that these could be identified by MALDI-TOF (from subculture) or abbreviated methods for a cost of $0.50. The costs associated with Gram stains and subcultures of all positive blood cultures are not included in the cost analysis, as they are assumed to be required despite the implementation of MALDI-TOF identification for the purpose of identifying polymicrobial cultures and performing susceptibility testing. All costs are in U.S. dollars.

TABLE 2 Performance of MALDI-TOF identification of 61 monomicrobial blood cultures and 2 polymicrobial blood cultures with the Bruker Sepsityper

Total no.	Definitive identification	No. with MALDI score of:			No. (%) of identifications concordant
		>2.0	<1.7	>1.7	
Monomicrobial culures					
13	*Escherichia coli*	13	0	0	13 (100)
8	*Staphylococcus aureus*	8	0	0	8 (100)
5	*Bacillus*/*Paenibacillus*[a]	2	3	0	5 (100)
5	Coagulase-negative *Staphylococcus*[a]	3	1	1	4 (80)
6	*Streptococcus pneumoniae*/*Streptococcus mitis* group[b]	0	2	4	5 (83.3)
3	*Enterococcus faecalis*	3	0	0	3 (100)
2	*Pseudomonas aeruginosa*	2	0	0	2 (100)
2	*Streptococcus gallolyticus*	2	0	0	2 (100)
2	*Staphylococcus epidermidis*	0	1	1	2 (100)
2	*Propionibacterium acnes*	1	1	0	2 (100)
1	*Streptococcus constellatus*	0	1	0	1 (100)
1	*Corynebacterium* sp.[a]	0	1	0	1 (100)
1	*Salmonella enterica*	1	0	0	1 (100)
1	*Klebsiella oxytoca*	1	0	0	1 (100)
1	*Klebsiella pneumoniae*	1	0	0	1 (100)
1	*Enterobacter cloacae*	1	0	0	1 (100)
1	*Candida albicans*	1	0	0	1 (100)
1	*Candida glabrata*	0	0	1	1 (100)
1	*Pantoea* sp.[c]	0	0	1	1 (100)
1	*Streptococcus dysgalactiae*	1	0	0	1 (100)
1	*Staphylococcus warneri*	1	0	0	1 (100)
1	*Staphylococcus lugdunensis*	1	0	0	1 (100)
1	*Rothia mucilaginosa*[a]	0	0	1	0 (0)
61	Total	42 (68.8)[e]	10 (16.4)[e]	9 (14.8)[e]	58 (95.1)
Polymicrobial cultures		MALDI-TOF result (score)			
1	*Enterococcus faecalis, Pseudomonas aeruginosa*	*Enterococcus faecalis* (2.021)			
1	*Fusobacterium* sp.,[d] *Veillonella* sp.	*Fusobacterium necrophorum* (1.615)			

[a] Isolates deemed contaminants were not further identified or characterized beyond the genus or group level unless required to rule out pathogenic organisms.
[b] Inability to accurately differentiate *S. pneumoniae* from other members of the *S. mitis* group is a recognized limitation of MALDI-TOF.
[c] Unable to identify to the species level by 16S sequencing. The closest match was *Pantoea septica*.
[d] Unable to identify to the species level by 16S sequencing. The closest match was *Fusobacterium necrophorum*.
[e] Percentage of the total.

RESULTS

Sixty-one positive monomicrobial cultures and two polymicrobial cultures were identified in this study (Table 2). Patients with multiple cultures taken as a set positive for the same organism(s) were only counted once for the study. Twelve isolates were deemed contaminants on the basis of established laboratory criteria (five of *Bacillus* sp., five of coagulase-negative *Staphylococcus* sp., one of *Corynebacterium* sp., and one of *Rothia mucilaginosa*). Overall, 42 (68.8%) of the isolates had high-confidence identification scores (>2.0, identification to the species level), 10 (16.4%) had good confidence scores (>1.7, identification to the genus level), and 9 (14.8%) had low scores or no peaks. Regardless of the MALDI-TOF identification score, 58 (95.1%) of 61 monomicrobial cultures were concordant with the final identification. Specimens that were considered discordant included: one isolate of *R. mucilaginosa* that failed to produce a spectrum on repeated attempts to perform the analysis directly from the positive blood culture, one isolate of *S. mitis* which was identified as *Lactobacillus sharpeae* (score, 1.222) by MALDI-TOF, and one isolate of coagulase-negative *Staphylococcus* that was identified as *Neisseria subflava* (score, 1.207) by MALDI-TOF. All of these isolates were correctly identified by MALDI-TOF from subcultures. All organisms with confidence scores of >1.5 were concordant with definitive identification. Table 2 summarizes the performance of MALDI-TOF compared to definitive identification. For the two polymicrobial cultures, one of the organisms present was correctly identified by MALDI-TOF, whereas the other was not identified from the direct blood culture. In both cases, the two organisms present were identified correctly from subculture.

The mean turnaround time for the conventional identification was 40.9 h (95% confidence interval [CI], 34.8 to 46.9 h), and that for MALDI-FOF identification was 6.6 h (95% CI, 5.2 to 8.0 h) ($P < 0.0001$) in the ideal situation where all organisms, including polymicrobial cultures, could be identified by MALDI-TOF, and 14.4 h (95% CI, 9.0 to 19.7 h), $P < 0.0001$ in the more practical situation where organisms with inadequate identification scores, polymicrobial cultures, and members of the *S. mitis* group continued to require conventional identification or identification from subcultures.

Excess costs (or savings) associated with the procedure over conventional methods, assuming that Gram stains and subcul-

TABLE 3 Cost estimates for conventional identification of blood culture isolates in three scenarios[a] versus MALDI-TOF Sepsityper identification[b]

Conventional ID scenario	Cost (U.S. dollars)/positive culture			
	Avg	Sepsityper	Conventional ID for MS failure[c]	Net
Low cost	1.58	5.15	0.07	3.64
High cost	5.50	5.15	0.07	−0.28[d]
Best estimate	1.98	5.15	0.07	3.24

[a] Low cost, high cost, and best estimate. See text for details.
[b] Estimates assume a typical distribution of isolates (see Table 1).
[c] Assumes 14.8% failure to identify with Sepsityper and cost of conventional identification is $0.50 per failed identification. See methods for details.
[d] Net savings.

tures continue to be performed and a 14.8% Sepsityper failure rate requiring conventional identification (see Materials and Methods) are presented in Table 3.

DISCUSSION

With a cutoff score of 1.7, the Sepsityper and BioTyper MALDI-TOF correctly identified 85.2% of the monomicrobial blood culture isolates tested. This compares favorably with data from others using in-house extraction systems for blood culture analysis which have reported correct identification rates ranging from 67 to 100%, with higher success rates for Gram-negative organisms than for Gram-positive organisms (11–13, 15–17). Similarly, a recent study by Buchan et al., also using the Sepsityper system, reported that 85.5% of the blood culture isolates tested were identified directly from blood cultures with better performance for Gram-negative organisms (3). Like others who have reported on the use of MALDI-TOF directly from positive blood cultures, we observed that cutoff values could be lowered somewhat without compromising accuracy (3, 10, 20). If a 1.5 cutoff were used, 54/61 (88.5%) isolates were correctly identified and none were misidentified. Our findings further support that the cutoff score for confident identification could be lowered when MALDI-TOF is applied directly to positive blood cultures. Recently, the manufacturer has developed a new software tool for the analysis of blood culture spectra that uses a lower cutoff value (Bruker-Daltonics, personal communication). It was noteworthy that of the 18 isolates with scores lower than 2.0, 10 (55.5%) were organisms typically considered culture contaminants, 5 (27.8%) were members of the *S. mitis* group, and 3 (16.7%) were presumed pathogens. If typical contaminants and members of the *S. mitis* group are excluded from the analysis, 36/39 (92.3%) cultures were correctly identified with scores of >2.0. This suggests that the performance of MALDI-TOF from positive blood cultures using the manufacturer's database is better with definitive pathogens than with presumed contaminants. Lower scores from presumed contaminants may be a result of lack of sufficient database entries for these organisms, an insufficient amount of proteomic material in these cultures resulting from the relatively low organism counts seen in contaminated cultures or competing protein material from blood components. Since the system has been shown to perform very well with pure cultures of several species of coagulase-negative staphylococci (7, 14), it seems somewhat more likely that the low scores obtain for these organisms in our study were due to either low organism counts, as has been described by others (6), or competing proteins from blood.

As observed by others (13, 17, 18, 23) and noted in the limitations of MALDI-TOF reported by the manufacturer, members of the *S. mitis* group, including *Streptococcus pneumoniae*, did not produce very high scores (1.152 to 1.734). Despite these low spectral scores, the identification was correct in 5/6 (83.3%) cases. In one case, an *S. mitis* isolate was identified as *L. sharpeae* with a score of 1.222. Notwithstanding the apparent accuracy, the manufacturer does not endorse the use of the instrument to distinguish between members of this closely related group and it seems prudent to report isolates that meet identification criteria as "*S. pneumoniae*/*S. mitis* group" pending additional identification tests such as bile solubility.

For polymicrobial cultures, MALDI-TOF correctly identified one organism in each culture on both occasions. The second organism was not present in the top 10 best spectral matches in either case. Polymicrobial cultures have been shown to typically yield a single identification by MALDI-TOF (3, 13). However, others have reported that it may be possible to identify multiple organisms with different Gram stain reactions (e.g., Gram-positive cocci and Gram-negative rods) in a single specimen by comparing the spectrum generated to two or more different libraries of organisms grouped by unique Gram stain reactions (9). Alternatively, additional organisms in a polymicrobial culture could be identified from subcultures on solid media. The difficulty in identifying polymicrobial cultures directly by MALDI-TOF underscores the importance of continued reliance on Gram stains and subcultures of positive cultures for definitive identification and therefore optimal patient care (10, 12).

Not surprisingly, turnaround time was greatly improved by using MALDI-TOF to identify organisms directly from positive blood cultures. We observed a mean reduction in turnaround time of 34.3 h, assuming the ideal situation of definitive identification of all isolates by MALDI-TOF and 26.5 h in a more practical scenario where organisms requiring further characterization were identified from subcultures. These observations are similar to those of Buchan et al., who reported a range of 23 to 83 h faster for Gram-positive isolates and 34 to 51 h faster for Gram-negative isolates (3). Historically, the Gram stain of a positive blood culture provided the most critical piece of information for the management of bacteremic patients because it provides rapid information that can be roughly correlated to empirical antimicrobial treatment. Identification to the species level, although helpful for differentiating pathogens from contaminants (particularly important for Gram-positive organisms) and for tailoring antimicrobial therapy to the intrinsic resistance of certain pathogens (particularly important for Gram-negative organisms) often takes 24 to 48 h. Although not yet proven, rapid (<20 min) identification of both relevant pathogens and contaminating organisms from positive blood cultures may be beneficial for patient outcomes, particularly when organisms with intrinsic resistance (*Pseudomonas* sp., *Stenotrophomonas* sp.) are identified. Rapid identification of blood culture contaminants may also allow more rapid discontinuation of unnecessary antimicrobial therapy (26).

The operating costs associated with the implementation of MALDI-TOF identification are likely to vary significantly by laboratory, depending on the current methods used for identification and the success rate of identification directly from positive cultures. Although the operating cost of MALDI-TOF identification from isolated organisms is small in comparison to routine commercial biochemical panels, the cost of the Sepsityper kit for use in

positive blood cultures is comparable to that of commercial panels. Therefore, laboratories relying primarily on commercial panels for the identification of blood culture isolates are unlikely to observe a significant change in operating costs. However, laboratories using abbreviated methods or in-house panels may see increases in cost, up to $3.64 per positive culture. This includes the cost of the 14.8% of the isolates that could not be identified directly from the positive cultures and would require identification from subcultures. In this study, all isolates that could not be identified directly from the blood culture bottles were adequately identified by MALDI-TOF or simple biochemical tests (e.g., bile solubility) from the subcultures at minimal cost (less than $0.50 per identification). Overall, most laboratories implementing this technology are likely to see a marginal increase in costs associated with the identification of pathogens from blood culture bottles. However, these increased costs will be offset by the very significant reduction in operating costs for the identification of pathogens isolated on routine media in other areas of the laboratory.

This study has a number of limitations. First, although efforts have been made to create a range of costs associated with the identification of pathogens from blood cultures, these will vary because of a number of other factors, such as the exact distribution of typical pathogens isolated, the utilization of MALDI-TOF MS and conventional methods within the general work flow of the lab, the exact costs of abbreviated identification methods and panels, and the laboratory protocols in place after the implementation of MALDI-TOF technology. For example, laboratories are likely to vary in terms of the amount of confirmatory testing by traditional methods that is required in instances where the MALDI-TOF identification score is low or if certain organisms are isolated. Similarly, turnaround time will vary between laboratories for reasons of work flow and requirements for confirmatory testing. Because of the complexities in determining these costs and turnaround times, definitive assessments will ultimately require *post hoc* analysis in laboratories implementing Sepsityper technology.

In conclusion, the Sepsityper and Bruker BioTyper represent a rapid, accurate tool for the direct identification of organisms from positive blood cultures resulting in an increase in the operating costs associated with identification from blood cultures.

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